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Alteration of Circulating Antibody Response of Mice Exposed to 9-GHz Pulsed Microwaves

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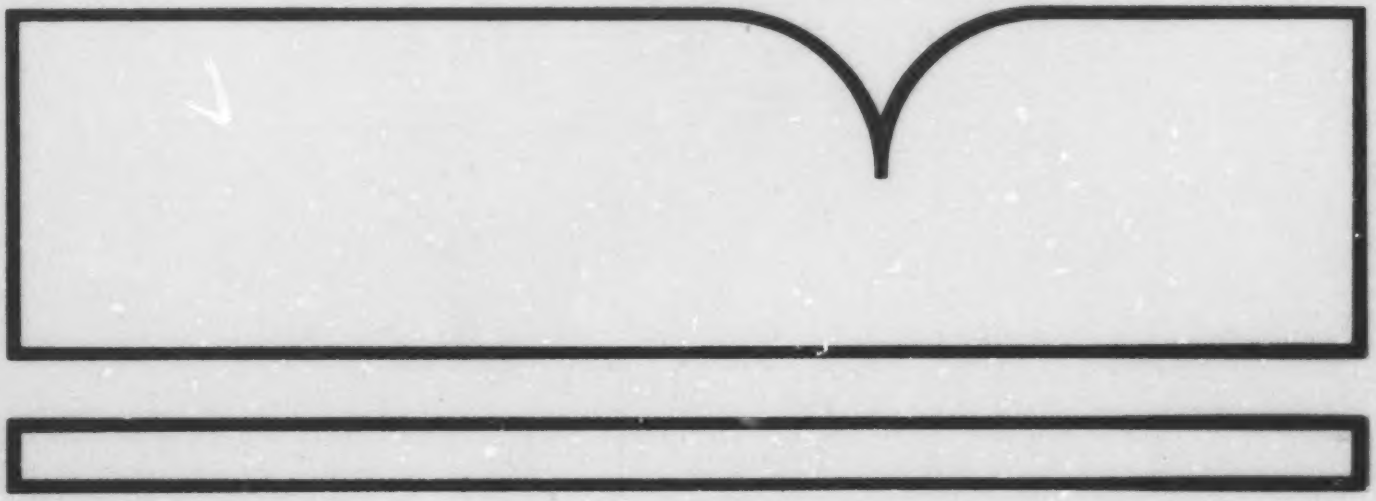

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16. ABSTRACT

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JOURNAL ARTICLE

Bioelectromagnetics 1:397–404 (1980)

Alteration of Circulating Antibody Response of Mice Exposed to 9-GHz Pulsed Microwaves

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A significant increase was observed in the circulating antibody titers of mice exposed to 9-GHz pulsed microwaves at an average power density of 10 mW/cm^2, two hours per day for five days compared with sham-irradiated animals. The mice were previously immunized with type III pneumococcal polysaccharide. Following irradiation, a portion of the immunized animals were challenged with virulent *Streptococcus pneumoniae*, type III. Ten days after challenge, mortality was essentially the same in the two groups, but during the ten day period, there was a noticeable increase in the survival time of the irradiated animals compared with the sham-irradiated animals, suggesting that the increased circulating antibody response afforded some degree of temporary protection to the animals.

Key words: antibody response, microwaves, immunology, 9-GHz pulsed radiation, infectivity, mouse

INTRODUCTION

Recently, there have been several reports indicating that microwaves may affect the immune system [Smialowicz et al, 1979; McRee, 1980; Stodolnik-Baranska, 1974; Czerski, 1975; Wiktor-Jedrzejczak , 1977; Huang et al, 1977]. Two of the studies dealt specifically with bone marrow-derived (B-cell) lymphocytes with Smialowicz et al [1979], reporting an increase in the mitogen-stimulated response of B lymphocytes and Wiktor-Jedrzejczak et al [1977] reporting a stimulatory effect on B-lymphoid cells of the spleen but a decreased plaque-forming cell (PFC) response to B-cell antigens.

The present study was conducted to determine if exposure of mice to 9-GHz pulsed microwave radiation would affect the ability of the animals to produce antibodies to type III purified pneumococcal polysaccharide (PPS), a B-cell antigen [Howard, 1971], and whether the microwave-induced stimulation of the immune system changes the degree of immunological protection as measured by subsequent challenge with Streptococcus pneumoniae, type III.

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MATERIALS AND METHODS

Experimental Procedures

The animals used in this study were CD-1 female mice 28–35 days of age weighing 21.5–27.5 g. Heparinized blood samples (200 λ) for hematology and preimmunization aintibody titers were obtained from the ventral tail veins of mice anesthetized with methoxyflurane. Red blood cell counts, white blood cell counts, hematocrit, and hemoglobin values were determined with a Model ZB1 Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.). Blood smears for white blood cell differentiation were stained with Wright's stain, Wilson's modification [Henry, 1974], and 100 cells were counted per slide. The mice were then held for one week prior to immunization.

The first six groups of four animals each were immunized by intraperitoneal (IP) injection of a killed bacterin of S pneumoniae, type III (No. 6303, American Type Culture Collection, Rockville, Md.), and the other groups were immunized with 0.25 μg (0.1 ml) of type III purified pneumococcal polysaccharide (PPS) (a gift from Eli Lilly and Co., Indianapolis, Ind.) by similar injection. The animals were then exposed in groups of four to 9-GHz pulsed microwave radiation in an anechoic microwave facility at an average power density of 10 mW/cm^2 (pulse repetition rate 970–1000, pulse width 1.0 μs, peak power 10W/cm^2) for two hours per day for five days. The calculated specific absorption rate was 3.3 mW/g (based on a multilayered sphere model [Weil, 1975]) or 4.7 mW/gm (based on a prolate spheroidal model [Durney et al, 1978]).

During irradiation, the mice were held in individual plastic containers (6 × 6 × 7 cm), described by Berman et al [1978], that were placed in polystyrene foam frames inside disposable, portable glove bags (50 × 50 × 30 cm, I^{2}R Corporation, Cheltenham, Pa), and were supplied with air at a flow rate of 0.014 m^3/min. The air temperature inside the bag (24.4 ± 0.1 °C) was maintained using an environmental control system described below. The sham-irradiated animals were held under identical conditions in a sham exposure chamber. Another group of four mice was injected with an equal amount of sterile saline and served as cage controls. On the second day of irradiation, rectal temperatures were taken before and after irradiation. The day after the last exposure (on day 6 after immunization), samples were again taken for hemagglutination and hematology analyses.

On the day of sampling for antibody titers, irradiated and sham-irradiated mice were challenged with an IP injection of an LD_{50} dose of virulent S pneumoniae, type III (9×10^3 organisms in 0.1 ml sterile saline). The organisms were reconstituted in trypticase soy broth from lyophilized samples prepared from a standard culture. Day of death was recorded for ten days after challenge.

For the hemagglutination procedure, sheep red blood cells (SRBC) in Alsever's solution were sensitized according to a modification of the method described by Askonas et al [1960] using 1 ml of purified polysaccharide (300 μg/ml) instead of a culture fluid. Antibody titers were determined by hemagglutination using doubling dilutions in V-bottom disposable plates (Cook Laboratory Products, Alexandria, Va.).

Microwave Exposure System

The X-band exposure facility (Fig. 1), which can be operated over a range of 8.5 to 9.6 GHz, is a free-space anechoic chamber and is designed to simulate radar exposures at a pulse repetition rate adjustable from 10 Hz to 6,000 Hz with a maximum duty cycle of 0.001 and a pulse width adjustable from 0.4 to 3.4 μs. Exposures were performed in the far field of a 10 dB standard-gain horn antenna at 1 m from the antenna, where a maximum

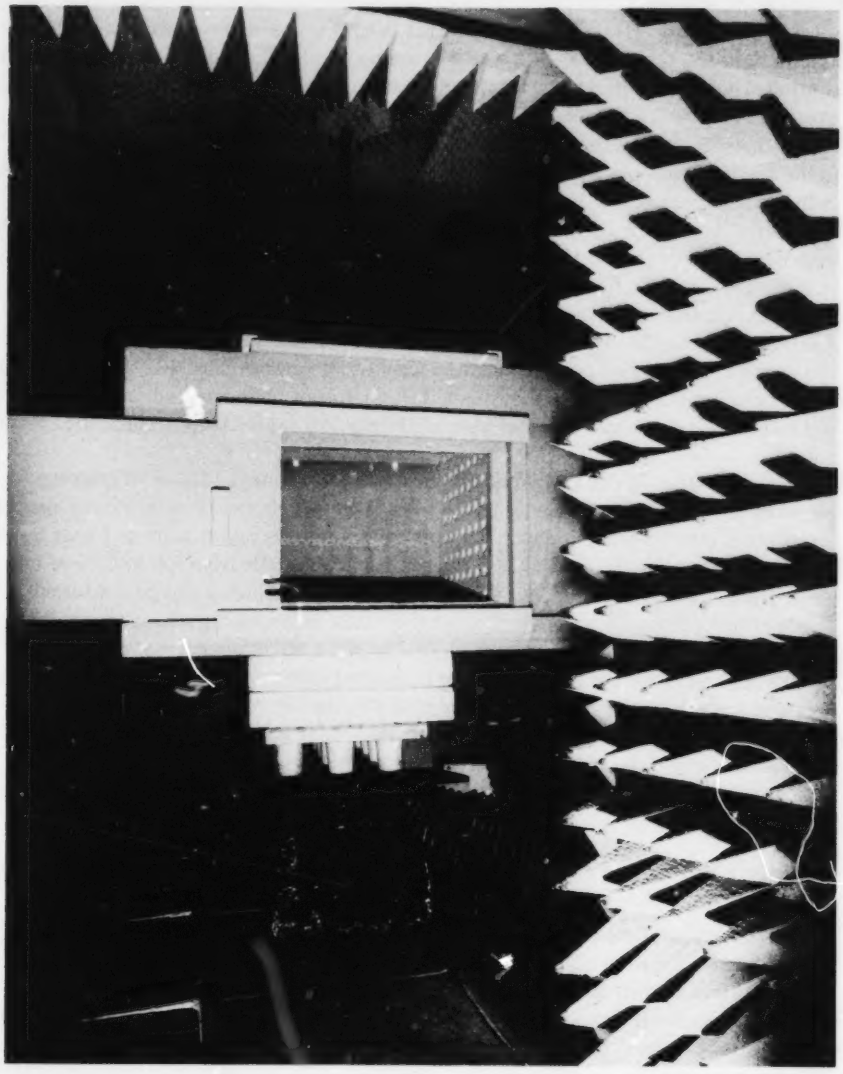

Fig. 1. The 8.5–9.6-GHz (X-band) microwave exposure facility with pyramidal RF absorbers on the walls and the door. The environmentally controlled exposure chamber is shown in the center.

average power density of 10 mW/cm^2 is obtainable with a field uniformity of ± 10% with a 20-cm-diameter circle.

The interior dimensions of the anechoic chamber are 2.7 X 2.7 X 2.6 m with floor blocks made of structural absorber (Model FFP-9, Microwave Control Products, Winchester, Ma.) and walls and ceilings of Model FFP-5 pyramidal radio-frequency (RF) absorber with Model FFP-8 absorbers in areas illuminated by the antenna radiation pattern.

A Styrofoam® (Dow Chemical, Midland, Mich.) animal exposure chamber with interior dimensions of 76.2 X 76.2 X 61 cm was constantly flushed with environmentally controlled air through ducting made of a neoprene-coated nylon fabric. Environmentally controlled air also was supplied to a similar sham-exposure chamber adjacent to the anechoic chamber was fed through insulated ducts to the animal and sham chambers.
to the anechoic chamber was fed through insulated ducts to the animal and sham chambers. Both temperature and relative humidity were regulated using three-mode proportional controllers. Average air velocity in the sham and animal exposure chambers can be adjusted from 3.0 to 10 m/min (± 0.1). Range of temperature control was from −10°C to +40°C (± 0.1C) and humidity from 20% to 80% (± 5%).

A block diagram of the exposure system is shown in Figure 2. Microwave power is generated by a Raytheon RK2J51 tunable pulsed magnetron rated at 40 kW peak power and driven by a modified pulse modulator (Applied Microwave Labs MH250) that contains a built-in adjustable pulse generator that allows control of pulse rate and pulse width. High voltage is supplied to the modulator by a separate power supply (Hipotronics, Inc., Model 820-50).

The RF output can be varied reliably only over a 6 dB range because of inherent operating characteristics of the magnetron tube. A waveguide switch network and associated components were installed to obtain a greater range of power adjustment. In Figure 2, the lower path represents the high-power range, which is adjustable from full to 0.25 of full power using the high-voltage power-supply control. The microwave energy is diverted through the 6 dB coupler and then through the rotary-vane variable attenuator to obtain lower power output. The attenuator is adjustable over a 60 dB range.

A portion of the available RF power is sampled with the 30 dB coupler for monitoring peak and average power, carrier frequency, and pulse width. A peak detection circuit is needed because of the extremely low average value of the detector pulse output. A 50 μamp panel meter is used with the peak detector circuit to indicate the null when the wavemeter is tuned. The pulse width is displayed digitally in microseconds with 10 ns resolution.

RESULTS

The hemagglutination titers were compared using analysis of variance to account for variability resulting from both the use of different batches of sensitized cells and the use of a bacterin in the first six groups of animals before PPS was obtained. The $\log_2$ titers among the microwave-irradiated mice were significantly higher than the titers among sham-irradiated mice (8.24 vs 7.88, $P = 0.0025$) as shown in Table 1. No detectable antibody titers were observed in the saline-injected controls.

Ten days after challenge, the mortality in the microwave-irradiated animals innoculated with virulent *S pneumoniae* was 47.2% (25/53), which was almost identical to the 50% (27/54) mortality rate found in the sham-irradiated animals. The difference between groups was not significant ($P = 0.46$) as determined by the one-tailed Fisher exact test [Siegel, 1956]. As can be seen in Figure 3, the greatest number of deaths in the microwave-irradiated animals occurred on day 6 following challenge, while the peak of mortality among sham-irradiated animals occurred at day 3. The mean values for the day of death were 5.58 ± 1.61 (SD) for the microwave-irradiated mice and 4.78 ± 1.37 (SD) for those sham irradiated. $P = 0.073$ for this difference using the Mann-Whitney U test. There were no survivors among the challenged saline-injected controls.

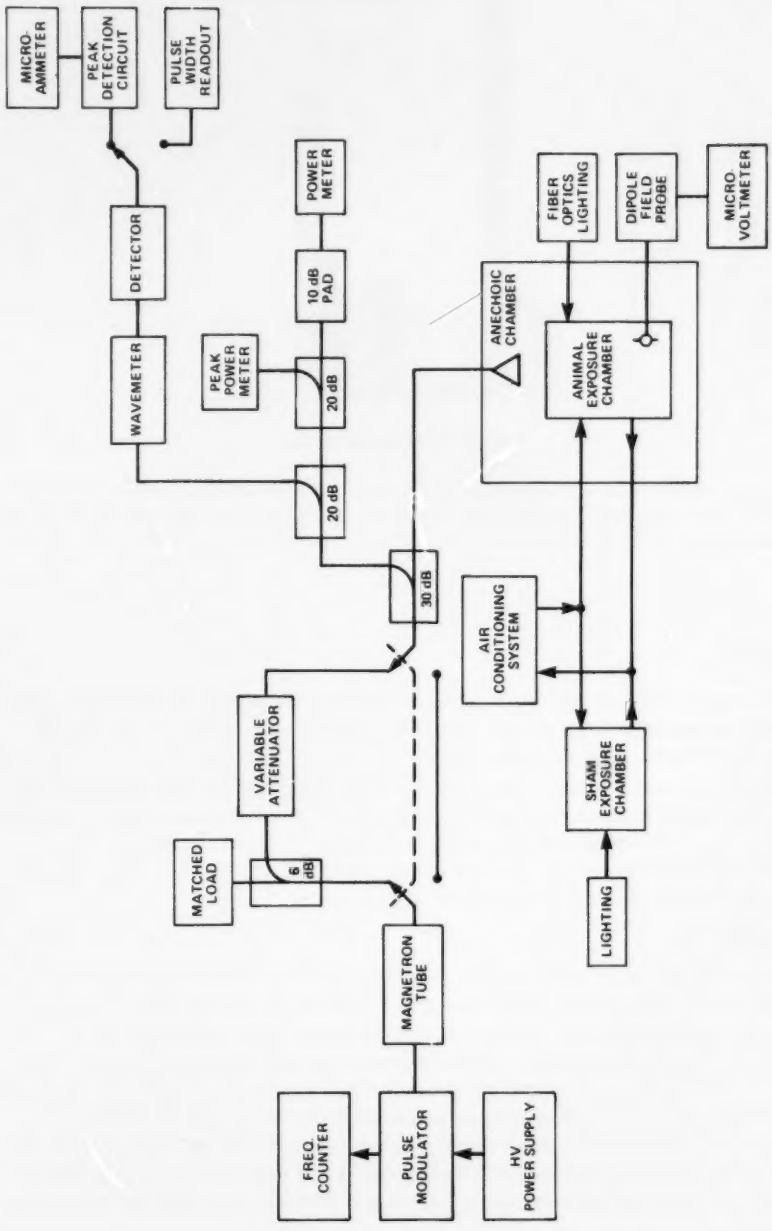

Fig. 2. Block diagram of the X-band exposure facility.

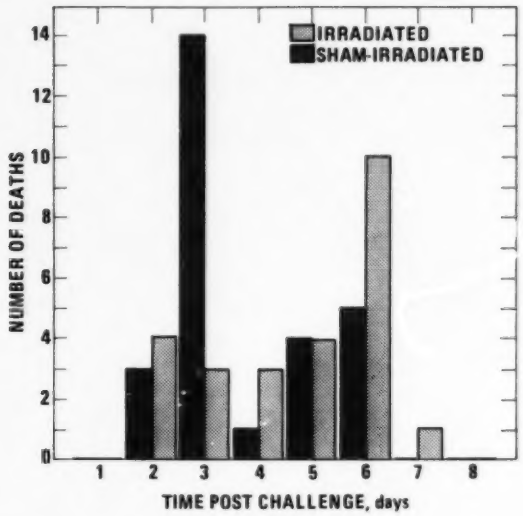

Fig. 3. Number of deaths per day following challenge with virulent *Streptococcus pneumonia.* The irradiated mice were exposed to 9-GHz pulsed microwaves, 10 mW/cm^2, for two hours per day for five days and challenged on the sixth day.

There were no statistically significant differences in the red blood cell counts, white blood cell counts, differential counts, hemoglobin values, or hematocrits between the irradiated and sham-irradiated animals (Table 2).

Rectal temperatures were taken before and after exposure on both the microwave- and sham-irradiated animals. Statistical analysis of the changes in rectal temperature during the exposure period did not show a significant difference between the microwave- and sham-irradiated animals (+0.08 ± 0.59 vs − 0.02 ± 0.72 °C, respectively), suggesting the animals were not subjected to a measurable thermal stress.

DISCUSSION

In the present study, no significant differences in the total leukocyte count or the differential count were found. Other investigators have reported leukocyte changes and/or differential count changes, but the reports have not shown any consistent pattern. Baranski [1971] reported a leukocytosis and a lymphocytosis in guinea pigs and rabbits exposed to 3000-MHz continuous-wave and pulsed microwaves at 3.5 mW/cm^2. Deichmann et al [1963] reported a lymphopenia and neutropenia in rats exposed to 24,000-MHz microwaves at 10 and 20 mW/cm^2, while Smialowicz et al [1979] reported no leukocyte changes in rats exposed to 2450-MHz CW microwaves at 5 mW/cm^2. In general, there do not seem to be any consistent changes in the pattern of leukocytes following microwave irradiation.

TABLE 1. Circulating Antibody Titers of Mice Exposed to 9-GHz Pulsed Microwave Radiation

Group	Number of mice	Titer ($\log_2$) ($\bar{x} \pm$ SE)
Irradiated	103	8.24 ± 0.11*
Sham-irradiated	100	7.88 ± 0.11

*P = 0.0025, F (1, 26) = 9.48.

TABLE 2. Hematology and Differential Results of Mice Exposed to 9-GHz Pulsed Microwave Radiation

Test	Microwave-irradiated n = 107 ($\bar{x} \pm$ SE)	Sham-irradiated n = 106 ($\bar{x} \pm$ SE)
Red blood cell counts (× 10^6/cu mm)	7.05 ± 0.11	7.04 ± 0.15
White blood cell counts (× 10^3/cu mm)	7.60 ± 0.35	7.46 ± 0.29
Hemoglobin (g/100 ml)	13.07 ± 0.12	13.19 ± 0.15
Hematocrit (%)	36.57 ± 0.50	35.74 ± 0.55
Lymphocytes (%)	83.5 ± 0.63	82.7 ± 0.82
Segmented neutrophils (%)	13.8 ± 0.58	14.0 ± 0.62
Banded neutrophils (%)	0.4 ± 0.10	0.4 ± 0.10
Eosinophils (%)	1.4 ± 0.15	1.6 ± 0.17
Basophils (%)	0.02 ± 0.01	0.02 ± 0.01
Monocytes (%)	0.9 ± 0.11	0.7 ± 0.11

The mice in this study apparently were not subjected to an appreciable thermal stress, as indicated by the lack of a significant increase in rectal temperature. If the increased immune response observed in this study was an effect of hyperthermia, it would have been the result of localized heat deposition or a thermal stress of a small enough magnitude that the animals were able to compensate for the added heat by thermoregulatory mechanisms.

The increase in circulating antibody titers observed in this study indicates that exposure to 9-GHz pulsed microwave radiation at an average incident power density of 10 mW/cm^2 affects the production of antibodies to S pneumoniae. The antibody titers were expressed as the reciprocal of the $\log_2$ so that an average increase of one represents a doubling of the number of circulating antibodies. The observed difference represents a 28% increase in the antibody titers of the irradiated animals. These results are similar to those of Smialowicz et al [1979], who reported an increased T- and B-cell response in rats following exposure to 2450-MHz microwave radiation at 5 mW/cm^2. Wiktor-Jedrezejczak et al [1977] also reported a stimulatory effect on B-lymphoid cells of mice exposed to 2450-MHz microwaves at an absorbed dose rate of 13 mW/g but found a decreased PFC response to B-cell antigens and no effect on T lymphocytes. These other reports and the results of this study suggest that B lymphocytes may respond to moderately low levels of microwave exposure.

The stimulatory effect of microwaves on B lymphocytes in animals exposed at power densities around 10 mW/cm^2, the present recommended maximum occupational exposure level, is an important finding; however, it is even more important to determine if such an effect is harmful, or perhaps beneficial, to the animal. One would expect an increased antibody titer, as seen in this study, to represent increased protection against S pneumoniae, since it has been reported that the resistance to challenge with virulent S pneumoniae parallels the antibody titers [Howard, 1971]. The virtually identical mortality between the microwave- and the sham-irradiated animals following challenge was unexpected. Analysis of the day of death showing an increased survival time suggests that there was a greater initial neutralization of the bacteria in the irradiated animals but that it was insufficient for complete recovery. These mortality patterns are similar to those seen in preliminary experiments in our laboratory to determine the LD_{50} of immunized mice. In these experiments, the majority of the animals challenged with doses greater than the LD_{50} died on the third day post-injection, while the peak of mortality for those animals given doses smaller than the LD_{50} occurred on the sixth day after injection.

These results demonstrate that there is a stimulation of the circulating antibody response of mice exposed to 9-GHz pulsed microwaves at an average power density of 10 mW/cm^2, and while the actual mortality was unaffected, there is evidence that the animals were able to survive a bacterial challenge for a longer period of time.

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